FINAL REPORT

19960272

ACCELERATED DEVELOPMENT OF VARIETIES OF SOLIN

September 2001
Funded by the Agri-Food Innovation Fund

Prepared by: University of Saskatchewan

AGRI-FOOD INNOVATION FUND

Canada

Saskatchewan

102-03853/

FINAL REPORT TO CANADA/SASKATCHEWAN AGRI-FOOD INNOVATION FUND

The Accelerated Development of Solin

Project Number 96000272

Final Report September 1, 2001

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EXECUTIVE SUMMARY

The primary objective of the program is to develop tools to hasten the development of new solin varieties and to generate new solin varieties. The objectives for this project were develop transgenic lines with altered oil composition, develop molecular marker technology, use this technology to develop molecular markers, screen an EMS mutagenized population for altered cyanogenic glucoside composition, and utilize traditional breeding methods to develop new solin lines. Three marker development technologies were examined. The amplified fragment length polymorphism (AFLP) technique proved to be the most reliable and useful. Markers were developed for linolenic acid content and yellow seed color. The use of these markers proved to be limited to the genotypes from which they were identified, a common problem with marker technology. Although numerous polymorphisms were identified between parents in the wilt resistance crosses, no marker was found that was linked to this trait. This is similar to the results obtained by researchers at AAFC (Morden MB Khalid Rashid person communication). Transgenic lines have been produced with six different constructs affecting oil composition: 35S/FATB1, Oleosin/FATB1, 35S/KAS, Oleosin/KAS, Oleosin/PCAT and Napin/FAE1. The FATB2 and KAS constructs affect medium chain fatty acid composition, with increases in C12, C14 and C16 fatty acids noted when these genes were expressed. The FAE1 resulted in the accumulation of C20 and C22 fatty acids. Plants harboring the PCAT construct showed significant increases in oleic acid content. A number of the transgenic lines accumulated 45 – 50% oleic acid compared to 15 – 20% in non-transformed controls. A mutagenized solin line was also screened for altered seed oil composition (funding from Saskatchewan Agriculture Development Fund). A number of mutants with increased oleic acid and stearic acid were identified and introduced in the solin breeding program. Finally, field selections have made for lines showing elevated levels of oleic acid. Crosses from EMS mutant lines that were tentatively identified as have reduced cyanogenic glucosides have been made and progeny analyzed by gas chromatography. A greater than 75% reduction in linamarin and linustatin was observed in these lines. Analysis of the 2000 Coop trial has identified two solin lines that show superior wilt tolerance and these were advanced into second year trials this summer. These lines have also now been included in most of our crosses in variety development. Increased oil content and palmitic acid levels has been realized in the breeding program for the high palmitic acid/ low linolenic acid varieties.

TECHNICAL REPORT

BACKGROUND AND OBJECTIVES

In western Canada, flax has traditionally been grown as an industrial oilseed crop. The recent development of solin (low linolenic acid flax varieties) will likely result in increased acreage seeded to flax for the human vegetable oil market. However, additional solin varieties will need to be developed before there is widespread cultivation in western Canada as there are currently only two varieties registered in Canada (Dribnenki et al., 1996; Can. J. Plant Sci. 76: 329). The requirements for registration of a new solin variety include a linolenic acid content of less than 5%, yellow seed coat and agronomic performance similar to traditional flax varieties. Thus, the development of new varieties will require a significant investment of time and labor.

Molecular markers, which can identify key traits necessary for new solin varieties (yellow seed, low linolenic acid, wilt resistance, yield etc.), will reduce both the time and resources needed for new variety development. The ability to transform crops with endogenous or foreign genes can also reduce the time for new variety development and produce plants that can fill niche markets. A major objective of this proposal was to develop molecular marker methodologies, which could be used in flax/solin, and identify molecular markers for yellow seed coat, linolenic acid content, and wilt resistance. We also were to produce transgenic flax lines with altered fatty acid composition.

The presence of cyanogenic glucosides in flax/solin reduces the value of the meal, thus the identification of lines which have low levels of these antinutritional compounds will increase the utilization of this crop. The CDC has a collection of EMS mutagenized flax lines that have been instrumental in the identification of numerous fatty acid alterations including the low linolenic acid solin phenotype. A portion of this collection was screened to identify lines with reduced levels of cyanogenic glucosides.

MOLECULAR MARKER ANALYSES:

Analysis of Yellow Seed Coat

Fifteen F2 populations of yellow x brown seed crosses were produced and evaluated. These populations were bulked and screened using both RAPD and AFLP techniques. In total 800 UBC primers, 250 Operon™ primers and 64 AFLP primer combinations were screened. A large number of polymorphisms were detected, most using the dominant yellow seed coat allele YSED18.

One linkage to the recessive YSED22 allele was reported in the 1998 report. This marker is of limited utility showing approximately 15% recombination between the band and the yellow seed trait. Of greater concern is the lack of extension of this marker to crosses other than YSED22 x ED47.

Screening using AFLP primer combinations identified several linked polymorphisms (e.g. Figure 1). Six fragments showed tentative linkage between the dominant yellow allele (YSED18) or the brown allele in the cross. These fragments were cloned and primer sets designed. Screening with specific primers was conducted with each pair of primers. Two primer sets were found to segregate with the seed color trait. Figure 2 illustrates the screening DNA from 6 yellow and 6 brown seed F2 plants, along with the bulk populations from which the markers were identified, the YSED18 and ED47 parents with the 3L primer set. The primer set produced a fragment linked to the yellow seed trait. Primer set 3LF/3LR produces a DNA of approximately 600 bp.

```
Primer 3LF 5' - TCAGTCATACCATGATTGTCG -3'
Primer 3LR 5' - GGTAGAATGGGAAACGTAT - 3'

Primer 1SF 5' - GAATCTGTTGAATGAGCCC- 3'
Primer 1SR 5' - TTGCGAAGTCAAATGGT - 3'
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The 3L primer set showed close linkage (8% recombination) to the yellow seed trait with (39 of 42 yellow seeded plants had the fragment and 16 of 18 brown seeded plants lacked the fragment). The primer annealing temperature was 54°C (Figure 2)

The 1S primer set was identified from a YSED18 x ED55 cross. A recombination frequency between the 1S primer set and the yellow seed color was calculated to be 20%. Part of the high recombination frequency may be due to the annealing temperature of 49°C, which may have increased the number of fragments amplified in the brown seeded plants. Also several of the plant showed more than a single PCR product. All other primer sets tested failed to amplify a fragment that segregated with the screened trait.

Figure 1: AFLP analysis of YSED18 x ED47 F2 progeny. DNA was bulked based on seed color – yellow (y) or brown (b) of the homozygous progeny. Circled bands were excised from gel, cloned and used for further marker development.

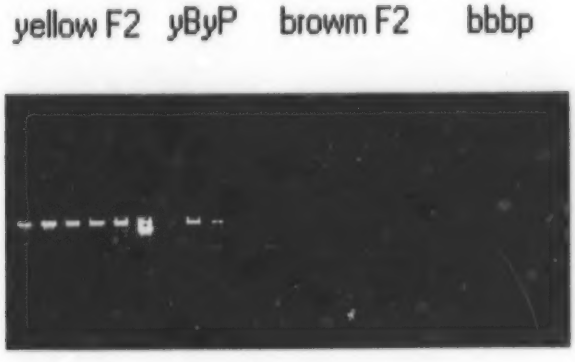


Figure 2: PCR reaction products using the 3LF/3LR primer set. DNA analyzed was from 6 homozygous yellow F2 plants, bulked yellow (yB), YSED18 parent (yP), 6 brown F2 plants, brown bulked (bb), and the ED47 parent (bp).

Markers for Wilt Resistance

A variety of populations segregating for wilt resistance were screened using both RAPD and AFLP screening methodologies. A total of 29 crosses between wilt resistant and wilt susceptible western Canadian flax/solin varieties were evaluated at the Preston Avenue flax disease nursery during the summers of 1997 – 2000 (Table 1). The evaluations were completed on a series of F3 lines and selection made based on relative resistance scores when compared to the original parents. Harvesting leaf material from 10 plants of each F3 line, extracting and combining the DNA from this material, developed the initial bulk populations. An aliquot of DNA from these initial bulked populations was then mixed with DNA from similarly scored resistant or susceptible F3 lines from the same cross.

Table 1 Table 1: Flax/solin crosses evaluated for wilt resistance at the Preston Avenue Disease Nursery and evaluated for marker studies

Flanders x ED66	Flanders x F94197	Flanders x B95042
Flanders x YSED2	S95407 X YSED2	S95407 x S96216
ED66 x YSED2	Bison x S95409	S95407 x YSED2
Bison x YSED2	F94197 x YSED2	B95042 x S95407
S95407 x S95409	S95409 x S96216	F94197 x S95407
S95409 x S96140	ED66 x S95407	S96216 x YSED2
S95409 x YSED2	ED66 x B94042	S96140 x YSED2
Flanders x S95407	ED66 x F94197	S96140 x S96216
Bison x S96216	Bison x S96140	Bison x S95407
B95042 x YSED2	S95407 x S96140	

The second population that was screened was a collection of 14 extreme types from the USDA flax germplasm collection. These lines were evaluated in 1998 and 1999 at the Preston Avenue wilt nursery. Although these populations were screened individually and as bulked populations, the results obtained in the disease nursery were not consistent with the ranking given these lines by the USDA. Of particular concern was the Bison seed that was obtained. This is a resistant variety that we use as a check in the nursery, however, the sample obtained from the USDA showed moderate susceptibility. Although this population was screened using the RAPD technique, given the questions raised by the nursery results, the samples were discarded and no further analyses were conducted.

The third group of plants evaluated was a recombinant inbred population obtained from Oregon State University. These lines are derived by single seed decent from an original cross of E1747 x Viking. The E1747 line is a wilt susceptible, low linolenic acid oilseed flax that was originally obtained from the CDC. Viking is a wilt resistant, high linolenic acid fiber flax. This population was seeded into the Preston Avenue wilt nursery and evaluated for wilt resistance. Resistant and susceptible lines were bulked and the DNA used for RAPD and AFLP analysis.

Using both the F3 and the recombinant inbred populations, numerous polymorphisms were identified between the two bulk populations. Figure 3 shows an AFLP gel of the recombinant inbred analysis. Numerous polymorphisms were also found between any parent combination. However, with one exception none of the polymorphisms identified in either the bulks or the parent plants showed linkage to the trait when individual lines or plants were screened using the same primer sets. One loosely linked RAPD polymorphism was identified to the F94197/S95407 cross (see 1998 report). However, sequencing the cloned fragment failed to yield an acceptable primer pair to develop PCR based marker with. The primer pairs tested all amplified fragments in all samples.

These results are similar to those obtained at the AAFC station in Morden MB. The most likely explanation for the lack of success is due to fact that segregation of the trait is still occurring in an F3 population. However, until an individual plant based assay can be developed this is the only logistically feasible way to develop marker populations. It would require a huge time and money investment to develop a series of near isogenic lines segregating for this single trait (F7 or F8 population would be required).

Fatty Acid Marker Development

We have completed crosses between S95407 x CDC Normandy to obtain marker populations segregating for the Δ15 desaturase genes. Previous work has indicated there are two Δ15-desaturase genes in flax, and all alleles appear to be expressed. Linolenic acid (ALA) content in the seed oil is the result of additive gene action. After gas chromatography analyses of the F2 plants, selections were made based on ALA content. Plants that showed 5% or lower ALA were bulked as a low population while those with ALA levels greater than 50% were bulked as the high population. An additional population was also developed which contained plants with non-segregating ALA contents of 25 – 35%. This population was classified as intermediate. We also evaluated an F7 recombinant inbred population for fatty acid composition. However, an insufficient number of lines were identified as being either low or high to create a marker population from this collection.

The three F2 bulk populations were analyzed using RAPD and AFLP primers. A number of polymorphisms were identified with RAPD primers at the bulk population level, but none demonstrated any linkage when screened against the individuals that made up these populations. AFLP analysis also identified a number of polymorphisms (e.g. Figure 3). Several of these polymorphisms did show linkage to either the high or low allele and the corresponding AFLP fragments were cloned, sequenced and primers designed. In all but one case the use of the primers did not result in the development of a usable marker. The TBF/TBR primer combination also failed to generate a linked marker when initially screened . The amplified fragments from both populations using this primer set were cloned, and the sequences from these two fragments were aligned. The presence of a HindIII site was found in the DNA from the high ALA population. Digestion of DNA sample prior to adding the primers revealed a linked marker after PCR (Figure 4).

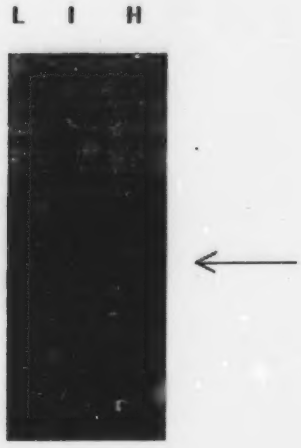


Figure 3: AFLP analysis of Flax DNA bulked on the basis of linolenic acid content. Bulk samples were made by pooling DNA from plants that were either low linolenic acid (L), high linolenic acid (H) or intermediate (I) in linolenic acid content.

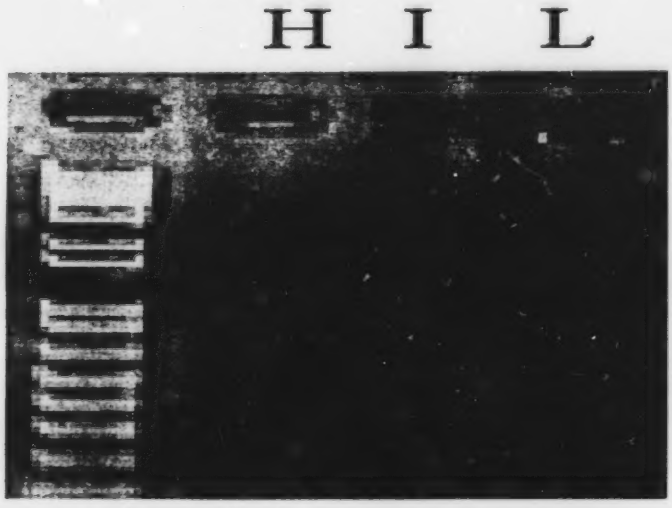


Figure 4: Agarose gel of PCR products from TB primer set. DNA was digested overnight with HinDIII and then analyzed by PCR. A 300 bp product was detected in DNA isolated from plants showing the low and intermediate ALA phenotype

The sequence of the TBF/TBR primer set is shown below. DNA is digested overnight with HindIII prior to PCR with the TB primers.

TBF 5′ ACATGTTTCCAACTGCCTG 3′
TBR 5′ TGCGCTCTTCCAATAGCAAA 3′

PRODUCTION OF TRANSGENIC PLANTS

Solin Plants Expressing Genes For Medium Chain Fatty Acid Accumulation

The *Cuphea wrightii* medium chain thioesterase (BFAT2) and the β–Ketoacyl Synthase (KAS) genes are required for the incorporation of medium chain fatty acids into the seed oil in the *Cuphea* species. These genes were used to transform the high palmitate (PA) containing solin line, HPED6. Initial transformations with both genes utilized the CaMV35S promoter. In both cases accumulation of C12 and C14 fatty acids was noted, as was an enhanced level of PA. Both constructs also had pronounced effects on plant morphology, which was attributed to the ectopic expression of these genes in the non-seed tissues. Both genes were reintroduced into the HPED6 background under the regulation of the seed specific oleosin promoter from *Arabidopsis*.

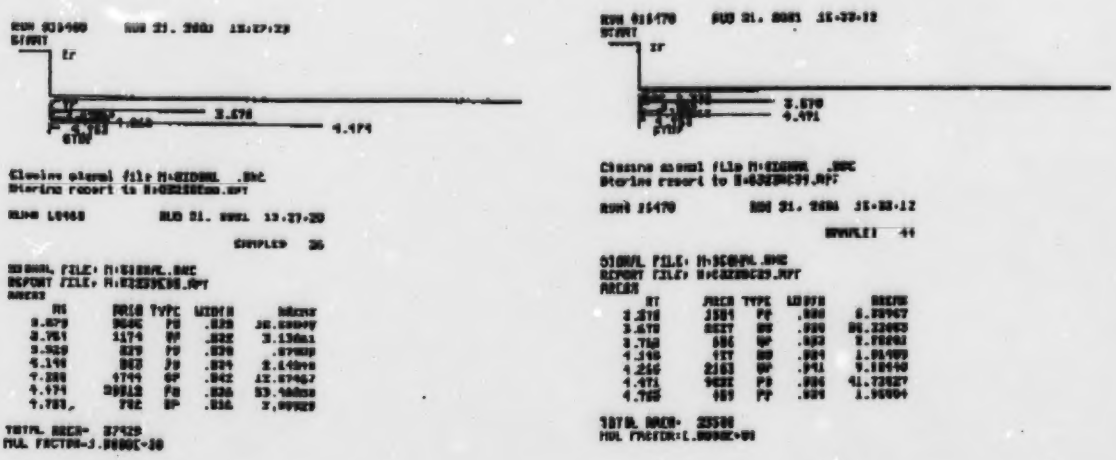
Fourteen independent 35S/BFAT2 lines were grown at Kernen Research Farm during the summer of 2000 in the transgenic plant section. Plant leaf tissue was harvested during the growing season and Southern blot analyses were conducted to confirm presence of the transgene. Relative to the HPED6 checks that were planted, most of the transgenic plants were small and either did not produce seed or yielded very poorly. Date of flowering was not significantly different but maturity was adversely affected by the transgene. The accumulation of medium chain fatty acids in the cell membranes of the non-seed tissues was likely the major factor contributing to these observations. However, several lines were fairly normal in appearance and yields were not drastically lower than the checks. Northern analyses indicated that the expression of the BFAT2 gene was greatly reduced in these lines.

Gas chromatography analyses of the seed oil indicated that under field conditions C12 and C14 fatty acids accumulated and an enhanced level of palmitic acid was also observed. The maximum level of C12 observed was 0.9% and C14 was 2.1% compared to no accumulation in the checks. Consistent with growth chamber experiments, C10 fatty acids were not detected. A slight enhancement in the PA level was also noted in the field-grown material. Plants that accumulated C12 and C14 fatty acids, had PA levels ranging from 18-22% compared to the 15- 17% PA content measured in the HPED6 checks.

Field results confirmed that expression of the BFAT2 gene under the regulation of the CaMV35S was detrimental to plant development. As a result both the BFAT2 and KAS genes were recloned into vectors under the regulation of the *Arabidopsis* oleosin promoter and used to transform the HPED6 line. The original transformation experiments were completed between January and March of 2000. Unfortunately we were unable to get any of these plants to root. This was a general problem in the lab as

none of our tissue culture material produced between January and June 2000 rooted. This problem was resolved and transformations were redone.

Fatty acid profiles are now complete for the majority of the plants transformed with the oleosin/TE and oleosin/KAS constructs. All plants were phenotypically normal with respect to general plant morphology and growth habit. This was in sharp contrast to many of the earlier 35S containing plants. Seed oil analysis was completed and half seed selections were made. In the T0 plants the level of medium chain fatty acids observed was lower than we had detected in the plants using the 35S promoter. Only trace (<1%) C12 fatty acids were measured and the C14 levels were 2 – 4% with both *Cuphea* genes. The reduced level of medium chain fatty acids probably reflects the fact that the oleosin promoter is not active early in seed fatty acid biosynthesis. As had been observed with the 35S plants, a large increase in palmitic acid was observed when these genes are expressed. Half seed selections from the BFAT transgenic lines have matured, and seeds analyzed. Again no phenotypic abnormalities were observed and all plants were fertile and showed excellent seed set. The level of medium chain fatty acids was also higher than was observed in the original T0 plants with levels of greater than 6% detected in a number of lines (Figure 5). These are likely homozygous plants, and this will be confirmed when the T2 plants are analyzed.



HPED6 T2 OLEOSIN:BFAT

Figure 5: Gas Chromatographs of seed oil from HPED6 and T2 Oleosin:BFAT plants. Plants were grown in environmental growth cabinets. Retention times are as follows 3.31=C12, 3.37=C14, 3.67=palmitic acid, 3.76=palmitoleic acid 4.14=stearic acid, 4.26=oleic acid. 4.47=linoleic acid, 4.75=linolenic acid. The trace peak at 3.82 seen in the HPED6 is an artifact that is likely the 2nd isomer of palmitoleic acid.

Fatty Acid Elongase Field Trial

The fatty acid elongase gene is required for the synthesis of C20, C22 and C24 long chain fatty acids. Thirteen lines of Napin/FAE1 transgenic plants were seeded at Kernen Research Farm during the summer of 2000. Preliminary data analysis revealed that there was no difference in the yield, flowering or maturity between the FAE1 containing plants and the CDC Normandy checks. Southern analysis was used to confirm the presence of the transgene in the field-grown material.

Analysis of the seed oil revealed only trace (<1%) amounts of C20 and C22 fatty acids in all but 1 line, which accumulated slightly more than 1% of these fatty acids in the seed oil. In growth cabinet raised plants, several lines showed an increase in the oleic acid level. However, this was not observed in the field raised plants with the oleic acids levels in the transgenic lines not significantly different than the checks. Another observation made in the growth cabinet was that linolenic acids were found to be 55-65% range in several of the transgenic lines compared to 50% in growth cabinet raised checks. In the field raised material, the linolenic acid content of the transgenic lines ranged from 57 – 63%, compared to the check which ranged from 54- 61%. While average linolenic acid content was higher in the transgenic lines, it was not statistically significant.

Production of a High Oleic Acid Flax

Three approaches were undertaken to increase the oleic acid content in solin: selection of high oleic acid material from our breeding lines, mutagenesis of solin breeding lines and expression of the PCAT gene under the regulation of the oleosin promoter.

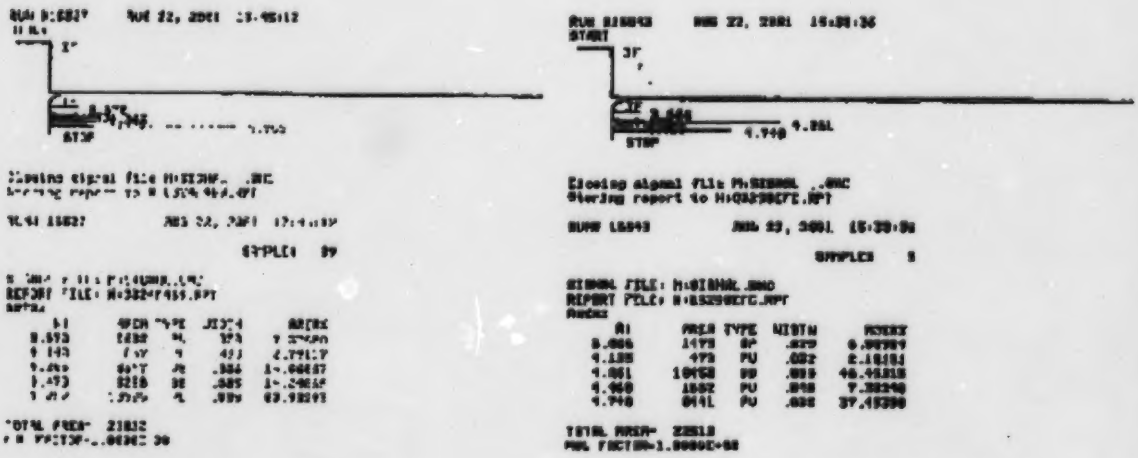
A number of the solin breeding lines that have been evaluated over the past six years were observed to have oleic acid levels greater than 20% of the seed oil in field grown material. This material was crossed and stable lines with oleic acid content of 25-30% have been developed. These lines are continuing to be developed and selections made for increased oleic acid.

A second approach was the use of mutagenesis to produce oleic acid mutants in the solin breeding line S95407 (this work has been partially funded by the Saskatchewan Agriculture Development Fund). Solin seeds were treated with 0.2% EMS and sown at the Kernen Research Plots. Screening of this material identified a number of mutants with elevated oleic acid content (oleic acid levels of between 32 – 42%). Half seed selections were then completed and these plants were raised to maturity in growth cabinets. The best mutant half seed selection accumulated 51% oleic acid. This line has been used to cross with the field oleic acid selections and with several other mutants.

In addition, the above crosses, large numbers of crosses were made between a couple of the original mutant lines identified, the high oleic acid field selections and each other. The results of these crosses are interesting. The crosses with the field selections resulted in an average increase in the oleic acid content (35 – 40%). The crosses made between each of the mutant lines resulted in a reversion to the non-mutant phenotype. As the parent plants in each case also showed the standard solin oil composition, it is

possible that an error occurred in seeding the pots with this material. Additional crosses involving other identified mutants are currently in the growth cabinets and will be harvested soon.

The last strategy to increase oleic acid content has been to transform plants with the soybean phosphatidylcholine acyl transferase (PCAT) gene. This gene was cloned downstream of the *Arabidopsis* oleosin promoter and was used to transform both CDC Normandy and the solin breeding line S93708. The PCAT enzyme catalyzes an exchange reaction between linolenic and oleic acids on the phosphatidylcholine molecule. This leads to a mass shift in the oleic acid available for seed oil synthesis. The seed specific expression of PCAT in CDC Normandy resulted in a dramatic shift in fatty acid composition. Oleic acid levels were increased to nearly 50% in a number of lines (Figure 5). There was a corresponding decrease in the linolenic acid pool in the seed oil. The expression of this gene on seed oil in the solin breeding line resulted in little or no change in the fatty acid profile. This result strongly supports the metabolic basis of the increase in oleic acid observed in the CDCNormandy lines – a mass flow shift in the linolenic /oleic acid pools



CDC NORMANDY **OLEOSIN:PCAT**

Figure 6: Gas chromatographs of seed oil from T1 oleosin/BFAT and CDC Normandy plants. Plants were grown in environmental cabinets. Retention times are as follows 3.67=Palmitic acid, 4.14=stearic acid, 4.26=oleic acid. 4.47=linoleic acid, 4.75=linolenic acid

Table 2 is a summary of fatty profiles of flax/solin lines developed during this project. The major shifts in fatty acid profiles are highlighted in red.

Table 2: Fatty acid composition of various flax/solin lines. (gc = environmental cabinet; MCFA = transgenic medium chain fatty acid lines; FAE = transgenic fatty acid elongase lines; FS = field selected oleic lines; Ol-EMS = oleic acid mutant lines, SE = stearic acid mutant lines)

Flax Type	>C16	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20+
Normandy – gc	0	5- 7	<1	3- 4	20-25	10-15	50-55	0
Normandy -field	0	5- 7	<1	3- 4	10-20	15-20	55-60	0
S95407/solin	0	5-7	<1	3- 4	15-20	55-70	1 -3	0
HPED6 – gc	0	18 - 26	2	3 -4	20-25	50-55	1- 3	0
HPED6 - field	0	15 - 17	2	2- 4	10-20	55-65	1- 3	0
MCFA – gc	2 - 9	25 - 40	2	3- 4	10-15	40-45	1 -3	0
MCFA - field	1 - 4	18 - 23	2	2 -4	15-20	50-60	1- 3	0
FAE – gc	0	5-7	<1	3- 4	15-25	10-15	50-65	1 – 2
FAE -field	0	5-7	<1	3- 4	15-20	15-20	55-65	1
PCAT Normandy	0	5-7	<1	2-4	35-50	10-15	35-40	0
PCAT S93907	0	5-7	<1	3-4	15-20	55-65	1-3	0
Oleic FS	0	5-7	<1	3-5	25-35	45-55	1-3	0
Oleic EMS - gc	0	5-9	<1	3-5	35-50	35-50	1- 3	0
Ol-EMS x FS	0	5-7	<1	3-4	30-40	45-55	1-3	0
Stearic EMS -gc	0	7-12	<1	10-12	20-30	40-50	1- 3	0
SExHPED6 -gc	0	30-40	3-5	3-10	15-30	30-40	2 -3	0

IDENTIFICATION OF LOW CYANOGENIC GLUCOSIDE FLAX MEAL:

Cyanogenic glucosides (CGs) accumulate in the seeds of flax and are present at high levels in the cotyledons of the germinating seedlings. In flax the two principle CGs are linustatin and neolinustatin are synthesized from the amino acids, valine and isoleucine, respectively. This conversion is a multienzyme process. In addition, a small amount of linamarin, which is an intermediate product in the linustatin pathway, is found in flaxseed.

Using combined modified methods of extraction and analysis indicated in methodologies of Kobaisy et al. (1996, J. Agric. Food Chem. 44: 3178), Bhatty (1993, JAOCS 70: 277) and Oomah et al. (1992, J. Agric. Food Chem. 40: 1346) we identified three EMS treated lines that showed reduced levels of cyanogenic glucosides on preliminary screening. Seeds from these lines were grown in growth cabinets and crosses between the three lines were completed. In addition, one of the lines was grown at Kernen Research Farm in the summer of 2000. Screening of seed from the harvested plants indicated that one of the original three lines did not have significantly reduced CG levels. The other two lines were reconfirmed as showing reduced CG content. The F2

progeny of the crosses between the two remaining lines (E523 & E477) showed a pattern of segregation for this trait consistent with simple Mendelian inheritance pattern (31:58:27 - high:intermediate:low) for CG content.

Table 3 : Cyanogenic Glucoside Content in McGregor Flax seed and selected EMS treated Flaxseed. Analyses were completed on defatted flaxseed and reported as mg of CG per g of meal.

Sample	Linamarin mg/g	Linustatin mg/g	Neolinustatin mg/g
McGregor	0.14	5.95	1.85
EMS523	0.19	3.70	2.39
EMS477	0.05	3.18	2.15
Line 1	0.08	2.88	1.92
Line 2	0.05	2.94	2.26
Line 3	0.06	3.07	1.89
Line 4	0.06	2.24	2.55
Line 5	0.05	2.51	2.00
Line 6	0.06	3.02	1.88
Line 7	0.05	2.95	2.06

Seven of these lines, plus the two original mutants and a McGregor flax sample were sent to POS analytical services for gas chromatography analysis of CG levels. The results from this analysis are shown in Table 3. The results indicate that a mutation has occurred in the valine to linustatin pathway in both of the mutant lines identified. A small increase in neolinustatin levels was seen in most of the samples. It is unknown whether this increase is significant or if a compensatory mechanism is operating in the plant to shunt additional product through the second CG pathway. The net result of this work was a reduction of >50% in linamarin levels and a 50 – 70% reduction in linustatin levels. A 0 – 15% increase in the neolinustatin levels was measured. However as the majority (~75%) of the CG content in flaxseed is synthesized via the linustatin pathway the decrease in this pathway more than offsets the increase in the alternate pathway. This material is currently being introduced in the flax/solin breeding program.

SOLIN BREEDING

High Palmitic Acid Solin Breeding

The initial HPLL (high palmitic, low linolenic) lines produced by the CDC were very late maturing and had very low seed oil concentration of around 35%. Plant breeding improved the oil content and maturity but the lines such as HPED6 still suffered from lower than desired palmitic acid levels. Industry wanted greater oil and palmitic levels. The breeding effort within this AFIF project was then directed at exploring if seed yield, maturity, oil content and palmitic acid levels could be improved.

In 2000 the last of the HPLL (high palmitic, low linolenic) lines developed within the AFIF project were grown in yield trials at Saskatoon, Indian Head and Canora,

Saskatchewan. The results of this experiment (Table 4) clearly show that further breeding improvements are possible in the HPLL background and that the high palmitic trait is not inherently associated with low oil content and poor agronomic performance. Line H0004 has a significantly higher seed yield and much higher oil content than HPED6 but is late maturing. However, while line H0005 has a seed yield equal to HPED6, it is significantly earlier maturing, has a very high oil content, higher palmitic acid concentration and a greater seed weight.

To date relatively little breeding effort has gone into this material. This research has shown that continued positive gains could be expected within this solin sub-group.

Traditional Solin Breeding

With the identification of wilt tolerant solin lines with good agronomic characteristics more crosses of these lines were made to superior flax parents. In 1999-2000 approximately 34,000 F2 seeds from these crosses were screened by the TBA (thiobarbituric acid) test for low linolenic acid and in 2000-2001 another similar number of F2 seeds were screened. About 5,500 early generation lines were raised at the Kernen Farm in 2000 and selections were made from these. In 2001 this is expected to rise to over 7,000 early generation lines. This material will provide a solid genetic base for future breeding and improvements of solin.

There were four CDC solin lines in the 2000 Solin Flax Cooperative Test. Two of these will be advanced into the second year of testing. SP2099 was the highest yielding line in the Black Soil Zone and while SP2100 did not have a high seed yield it was much earlier maturing. These two lines may find a niche in western Canada and certainly are being utilized as parental material.

Advanced testing of CDC solin also occurred at 8 locations in western Canada (Table 5). This is the largest group of wilt tolerant breeding lines that have so far been developed at the CDC. Some of these, such as S99036, have a combination of good seed yield, oil content and high linolenic acid content. The best of these lines are being advanced to the 2001 Solin Flax Cooperative Test.

Table 4. 2000 high palmitic solin yield trial grown at Saskatoon, Indian Head and Canora, Saskatchewan

Line	Pedigree	Yield (kg/ha)	1000 seed wt.(g)	Maturity score*	Oil (%)	Fatty Acid Palmitic (%)	Palmitoleic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)
HPED6	HPED6	1027	4.5	2.8	38.3	19.3	1.8	3.1	13.7	59.0	2.2
H97030	67/1747//VIMY/1747///1536/1747	948	4.9	3.2	36.8	19.7	2.2	2.3	12.7	57.5	4.5
M97046	1747/1929//67/1747///E1306	896	4.7	3.2	41.1	19.9	2.1	2.7	13.5	55.9	5.0
H98018	HPED-3/92F4 2605SPS	1071	4.8	3.3	40.4	18.6	1.7	3.1	13.6	60.3	2.0
H98006	HPED-3/92F4 2605SPS	1194	5.0	3.7	39.9	18.4	1.6	2.8	15.0	59.5	2.0
H99001	HPED-3/92F4 2605SPS	863	4.9	3.5	40.0	19.1	1.8	2.5	13.3	60.1	2.4
H99002	HPED-3/92F4 2605SPS	891	4.7	3.3	38.8	19.3	1.9	2.8	13.8	59.3	2.1
H00001	HPED-5/F93525	878	4.9	2.2	40.5	20.2	2.0	3.2	14.1	57.6	2.2
H00002	HPED-5/F93525	995	4.9	2.7	41.7	19.3	1.7	3.3	14.8	58.0	2.3
H00003	HPED-5/F93525	1009	4.8	2.7	41.0	18.8	1.4	3.4	15.2	58.2	2.1
H00004	HPED-5/F93525	1176	4.6	3.3	42.2	19.1	1.6	3.6	15.1	57.5	2.3
H00005	HPED-5/F93525	1080	5.0	2.3	42.7	20.6	1.9	3.2	14.7	56.6	2.1
H00006	HPED-5/F93525	1127	4.8	3.5	42.6	19.4	1.7	3.1	14.4	58.2	2.5
H00007	HPED-5/F93525	852	4.5	2.2	42.7	20.2	2.0	3.3	15.6	55.9	2.2
Mean		1000	4.8	3.0	40.6	19.4	1.8	3.0	14.2	58.1	2.6
CV		19	5.4	19.1	1.6	1.8	4.9	4.9	3.1	2.3	53.1
LSD		118	0.3	0.4	0.7	0.4	0.1	0.2	0.5	1.4	1.5

* 1=early, 5=late

Table 5. 2000 solin advanced yield 1 trial grown at 8 locations in western Canada.

Line	Pedigree	Yield (kg/ha)	Yield rank	1000 seed wt.(g)	Maturity (days)	Oil (%)	Linoleic acid (%)	Linolenic acid (%)	Total saturates (%)
SP947	Linola947	1939	1	5.1	109.5	40.1	70.5	1.8	11.2
SP989	Linola989	1756	14	4.9	107.9	38.7	71.3	1.9	10.9
SP1084	Linola1084	1755	16	4.9	107.6	41.8	70.9	1.7	10.3
S99336	ED-52/F93691	1432	35	4.8	108.6	40.9	72.5	1.9	9.9
S99044	ED-47/YSED-18	1539	29	4.9	111.9	39.7	72.7	2.1	10.6
S99048	ED-47/YSED-18	1754	17	4.9	111.9	40.1	73.0	1.9	10.6
S99032	F91109/YSED-19	1797	11	5.4	109.1	40.5	72.3	1.8	9.8
S99026	92F4 2605SPS/YSED-19	1535	30	5.1	107.5	39.1	69.4	1.8	11.4
S99057	ED-47/YSED-18	1511	31	4.9	113.7	40.4	71.4	2.0	10.7
S99039	ED-47/YSED-18	1638	25	4.9	111.7	40.4	70.6	2.1	11.1
S99054	ED-47/YSED-18	1650	23	4.8	112.8	40.6	72.7	1.9	10.6
S99029	92F4 2605SPS/YSED-19	1585	27	5.0	108.0	41.0	71.5	1.9	10.8
S99036	F87005/YSED-19	1921	2	5.2	109.2	40.1	72.7	1.8	10.6
S99038	ED-28/FP906	1673	22	4.9	112.1	40.8	71.2	2.1	11.0
S99031	F91109/YSED-19	1650	24	5.1	106.5	39.7	71.2	1.8	10.0
S99103	ED-55/YSED-18	1758	13	5.4	111.0	39.1	70.8	1.8	10.8
S99086	ED-47/YSED-18	1806	9	5.2	108.9	39.5	72.2	1.9	10.9
S99090	ED-55/YSED-18	1753	18	5.3	108.8	39.7	70.6	1.9	11.0
S99085	ED-47/YSED-18	1824	6	5.3	110.1	38.9	72.2	1.9	11.0
S99106	ED-56/YSED-18	1601	26	5.6	109.1	39.7	71.8	1.9	10.9
S99121	ED-56/YSED-18	1813	8	5.6	109.9	39.7	71.8	1.9	10.8
S99116	ED-56/YSED-18	1756	15	5.1	109.6	40.9	73.0	1.8	10.7
S99102	ED-55/YSED-18	1717	21	5.4	109.0	38.5	71.3	1.8	10.8
S99096	ED-55/YSED-18	1885	5	5.2	109.9	39.6	70.9	1.8	10.8
S99105	ED-56/YSED-18	1332	36	5.6	107.2	39.4	71.6	1.9	10.8
S99123	ED-56/YSED-18	1504	32	5.3	108.2	40.6	72.4	1.8	10.7
S99077	ED-47/YSED-18	1455	34	5.0	112.0	40.5	71.9	2.0	10.9
S99179	ED-28/FP906	1498	33	5.0	114.6	40.7	71.3	1.8	11.0
S99149	F93652/YSED-18	1902	3	5.0	108.4	39.3	71.9	1.7	10.7
S99131	ED-56/YSED-18	1820	7	5.3	109.6	40.7	72.7	1.8	10.8
S99171	F93652/YSED-18	1725	20	5.4	108.2	39.2	71.5	1.9	10.4
S99150	F93652/YSED-18	1790	12	5.0	109.9	39.5	71.6	1.8	10.8
S99161	F93652/YSED-18	1734	19	5.1	108.8	39.9	71.0	1.7	10.5
S99167	F93652/YSED-18	1895	4	5.2	112.3	38.9	70.8	1.7	10.9
S99162	F93652/YSED-18	1803	10	5.0	109.1	39.2	71.4	1.9	11.0
S99172	F93652/YSED-18	1572	28	5.1	107.8	39.2	71.8	1.8	10.7
Mean		1697	0	5.1	109.7	39.9	71.6	11.2	10.7
CV		16	0	5.7	1.9	2.9	1.4	1.8	2.9
LSD		118	0	0.1	1.1	0.5	0.4	0.1	0.1

CONCLUSION:

Marker Development: Technology to develop markers in solin has been developed. Populations segregating for seed color and oil content have been created which can be used for screening with new technologies when they develop. A large commitment of time and labor will be necessary to establish marker population that are suitable for disease screening. A number of cross specific primer sets were developed within the time frame of this project.

Modified Seed Oils Solin plants with elevated levels of medium chain fatty acids and elevated palmitic acid have been produced through introduced of *Cuphea wrightii* genes. Although numerous developmental problem were noted with these lines, the seed produced was viable, indicated that these fatty acids do not have detrimental effects on seed development. This finding was reconfirmed with the analysis of lines expressing these genes under the regulation of the oleosin promoter. This promoter also eliminated the developmental abnormalities observed in the 35S containing plants.

Solin plants with elevated oleic and stearic acid have also been identified from EMS treated material. Work will continue to develop this lines and further enhance the accumulation of this fatty acids. The use of the soybean PCAT gene also generated plants with increased oleic acid.

Long chain fatty acid accumulation is below economic thresholds in flax with the expression of the *Arabidopsis* FAE1 gene. A small increase in the linolenic acid content in these lines may be significant and breeding with lines showing elevated linolenic acid may allow for further enrichment of this fatty acid in the seed oil.

Cyanogenic Glucoside Content: We have developed a reliable, cost effective assay for measuring CG content in the EMS flax population. A major reduction in the one CG pathway was achieved through this project. A total CG reduction of nearly 50% was noted in some of the lines. This material is being introduced into the flax/solin breeding program.

Solin Breeding: Breeding has resulted in the identification of solin germplasm with superior wilt resistance. The yield drag that was noted with the first two resistant lines (S95407 & S95409) appears to have been overcome based on 2000 Cooperative trials.

PERSONNEL

Dr. Ron Wilen	June 1997 –March 2001 (advisor after Sept. 99)
Ms. Shannon Froese	June 1997 – March 2001
Ms. Phyllis Mykota	June 1997 - December 1997
Ms. Natalyia Krasichynska	June 1997 - August 1997
Mr. Tim Dament	September 1997 – May 2000
Dr. Satender Hansra	October 1999 – August 2000
Ms Shelley Muir	April 2000 – April 2001
Ms Jill Wagner	April 2000 – August 2000
Ms. Zoe Lawman	May 2000 – April 2001
Mr. Fawad Ahmad	September 2000 – March 2001
Dr. Yousif Hormis	February 2001 – March 2001

EQUIPMENT

Compaq presario 4180 computer - serial # 6715BLP2066
Daytek monitor - serial # EWBOC15AB01
Hewlett Packard 820cxi printer - serial # SG73EIJ11P
Hewlett Packard 6100C scanner - serial # SG7BB140CK
Gilson pipetman (8; various sizes) serial # O63573A P55917A
N67818K J18312O
Q63572A J18325O
L23336O Q58091C

Kenmore freezer serial # 06386831FD
BioRad sequencing gel apparatus serial # 367RB04023
Hybaid Omn-E 96 well heated lid thermocycler serial # 6718
Techne Genius 96 well thermocycler serial#
Spectroline transilluminator serial # TR365
Maintenance/repair on Perkin Elmer 9600 PCR serial # 5593 (note it was cheaper to refurbish this machine than to purchase a new piece of equipment)
Computer software - site license for Microsoft office, DNAMAN, Corel Suite 8
Miscellaneous labware and shelving units

INNOVATION FUND ACKNOWLEDGMENT

Gordon Rowland (1998) "Variety Development" at Flax Opportunities 2000 (Saskatoon January 12th 1998)

Ron Wilen (1998) "The Future of Solin". at Flax Opportunities 2000 (Saskatoon January 12th 1998)

Ron Wilen, Tim Dament, Gordon Rowland (1998) "Developing Molecular Markers in Solin" abstract from Conference on Impact of Molecular Biology on Crop Production held Aug 30 - Sept 1, 1998 in Minneapolis MN.

Rowland GG, Wilen RW (1998) "New Trends in Linseed Breeding" *In* Bast Fibrous Plants Today and Tomorrow. FAO Rome pp. 32 - 37

Rowland GG (1999) New Varieties for Saskatchewan - presentation to the Western Canadian Flax Growers Annual Meeting - Saskatoon January 11, 1999.

Ron Wilen (1999) "Using Biotechnology to Develop New Flax Varieties." Department of Plant Science - Montana State University, Bozeman MT - (October 7, 1999).

Rowland GG, Hormis Y, Wilen RW (2000) Developing new flax varieties using mutagenic approaches. *In* Utilizing Mutagenesis in New Crop Development. FAO Rome (in Press)